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Published in:
Book of Abstracts, Sustain 2017

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Ashley, J., Feng, X., Halder, A., Zhou, T., & Sun, Y. (2017). Dispersive Molecular Imprinting of Proteins for the Production of Plastic Antibodies. In *Book of Abstracts, Sustain 2017* [H-2] Technical University of Denmark.

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Dispersive Molecular Imprinting of Proteins for the Production of Plastic Antibodies

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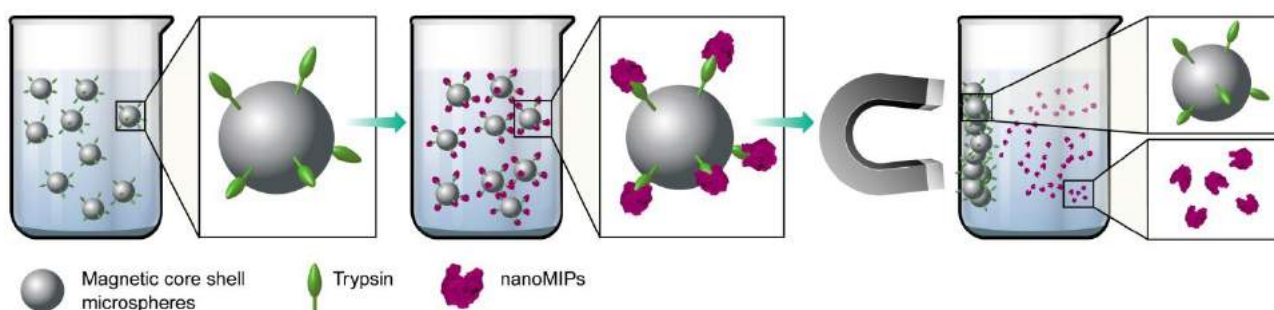
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The increased use of natural antibodies as affinity agents in both basic and applied research for the detection of proteins has resulted in a higher demand for the use of animals needed to raise these antibodies. As a result there is a push to reduce the dependency on animals in research through the development of synthetic alternatives. One area of increasing promise is in the development of nano based molecularly imprinted polymers (nanoMIPs) better known as plastic antibodies^[1]. These artificial receptors are formed by crosslinking functional monomers in the presence of a protein template to form nano sized polymers. After the removal of the template, a binding recognition site selective for the protein is left behind. Some challenges still remain to imprinting proteins such as difficulties in template removal, low yields and retention of the native structure of the protein during polymerization.

With this in mind, we set out to develop a new imprinting methodology which overcomes some of the limitations associated with conventional imprinting methods^[2]. The new approach termed “dispersive solid-phase imprinting” where the template protein is immobilized onto the surface of magnetic microspheres as a solid-phase which allows for the plastic antibody to be imprinted round the protein. This methodology demonstrates some attractive advantages over other solid-phase materials. The high surface to volume ratio of magnetic microspheres leads to increased rates of immobilization of protein and increased yields of plastic antibody per g of solid phase used. Unlike glass beads, magnetic microspheres do not suffer from abrasion due to their molecular sizes (600 - 700 nm) which allows for reaction mixtures to be dispersed throughout the mixture during the polymerization and in turn leads to increased solution-phase reaction volumes. The use of a magnetic core allows for the easy manipulation of the microspheres during the washing and elution steps with both reaction and purification steps being completed within 3 hours (**Scheme 1**). Using trypsin as a model protein, we developed plastic antibodies which were synthesized and characterized using TEM and dynamic light scattering demonstrating a size of about 209 nm. The plastic antibodies demonstrated a high binding affinity (2×10^{-7} M) and selectivity towards trypsin. Overall plastic antibodies could potentially replace the use natural antibodies in healthcare based applications, increase the shelf life of antibody based bioassays and medical diagnostic kits as well as reduce the use of animals in biological research.



Scheme 1: Overview of dispersive molecular imprinting.

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- [2] F. Canfarotta, A. Poma, A. Guerreiro, S. Piletsky, *Nat. Protoc.* **2016**, 11, 443–55.